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THE ROLE OF THE EYE AND CNS COMPONENTS IN PHOTOTAXIS OF THE ARROW WORM, *SAGITTA CRASSA* TOKIOKA

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Abstract

The arrow worm *Sagitta crassa* swims by a slow tactic behavior toward a light of moderate intensities and by a quick target-aiming upon sudden reduction in light intensity. The swimming stroke is achieved by a dolphin-type thrust. Microcautery of eyes, brain and ventral ganglion has shown that one-eyed worms can show the two kinds of photic responses and that specimens with both eyes or the brain damaged can survive, but fail to respond to light. Swimming itself, however, depends on the ventral ganglion being intact. These results indicate that the responses are mediated telophototactically through the eyes, and that no extraocular photoreceptors can be substituted for them. The eye structure which possesses one central pigment cell with seven depressions on four sides is fitted for the telophototaxis, enabling photoreceptive endings in the depressions to be stimulated to different degrees by light coming from one particular direction.

Introduction

Little is known about the sensory physiology of chaetognaths despite their great importance in marine zooplankton. To date, most research has come from morphological observations of the nervous system and sense organs (Hesse, 1902; Burfield, 1927; Kuhl, 1929; John, 1931, 1933; Eakin and Westfall, 1964; Horridge and Boulton, 1967; Bone and Pulsford, 1978; Ducret, 1978). Some physiological correlations are known in a few cases; the worms respond to moderate amplitudes of water borne vibration (Horridge and Boulton, 1967; Newbury, 1972; Feigenbaum and Reeve, 1977), touch (Bieri, 1966; Fraser, 1969) and light (Esterly, 1919; Spooner, 1933; Pearre, 1973; Goto and Yoshida, 1981). However, information on the mechanisms that link receptor and effector organs is scanty, and direct evidence for the role of the presumed receptors is usually lacking.

In a previous paper (Goto and Yoshida, 1981), two types of light responses were reported for the arrow worm *Sagitta crassa*: a) a slow tactic movement (ST) toward a light of moderate intensities effected by repeated hopping and sinking and b) a quick target-aiming (QTA) by which worms swam straight toward a light source either when the light beam was suddenly reduced or when a water borne vibration was applied. Both of these behavioral responses were thought to be telophototaxes. The present work demonstrates that such responses are executed through the eyes alone. Furthermore, information pathways from photoreceptors to effectors were investigated by cauterizing the eye, the brain and the ventral ganglion in various combinations.

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Materials and Methods

Arrow worms, *Sagitta crassa* Tokioka, collected by towing a plankton net were kept for use in aquaria at 17°C with an LD 12:12 cycle (Goto and Yoshida, 1981).

For selective microcautery, only healthy and actively swimming mature worms were used. Worms anesthetized in eugenol containing sea water (100 ppm) were gently placed on an agar plate. The tissues in question were cauterized instantaneously with a steel needle (electrolytically sharpened to 20 μm tip diameter) attached to a radio frequency microcauterizer (100 MHz, 1200 V). Microcauterized worms were returned to aquaria immediately and left for at least two hours. After experimentation, worms were fixed in 2.5% glutaraldehyde, embedded in epoxy resin (TAAB embedding resin), sectioned transversely and stained with toluidine blue. The extent of the cauterization was determined by light-microscopy.

Moribund or inactive worms were discarded. Behavioral experiments were done during the later half of the L-phase of the above cycle. The slow tactic behavior (ST) and quick-target-aiming (QTA) of the microcauterized worms were examined in a specially designed trough (Fig. 1). The trough, 12 × 12 × 4 cm, had a small compartment in one corner (upper right in the figure), which was separated from the main part by a plastic plate, 4 cm in length. Experiments were done in a dark room at temperature ranging from 15°C to 17°C.

ST's were examined in a sequence from a to c (Fig. 1). An arrow worm (shown by an arrow) was dark-adapted in the small compartment for 10 min (a). The partition sheet was lifted up gently (c) and two white light sources, L_1 and L_2 (each being a 100 V-300 W incandescent bulb) were turned on simultaneously. Each beam was collimated by a lens system and passed through an infrared absorbing filter (Toshiba IRA-25S). The intensity of each beam was measured in front of the trough wall by means of a radiometer (Japan Spectroscopic Co., Ltd. Model RMD-1) and was adjusted to become equal, 5.5 $\mu W/mm^2$, by neutral density filters (Toshiba). Worms that reached the wall facing either L_1 or L_2 within 10 min were assumed to have responded positively (pluses in Table II).

QTA's were examined in a sequence from b to c in Figure 1. A worm in the small compartment was illuminated for 10 min (b) with three light beams, L_1, L_2 and L_3, the intensity of L_3 being 70 $\mu W/mm^2$. The partition was then lifted gently and L_3 was simultaneously turned off (c). The resulting light intensity reduction (about 86%) was the stimulus to induce QTA. Worms which moved immediately toward either L_1 or L_2 by a series of repeated rapid swimming movements were given two pluses (++). One plus (+) was given to those which became simply more active; swimming for a short distance, changing body orientations, etc. A minus (−) indicates no response in both ST and QTA.

Instantaneous profiles of worms showing ST or QTA response were photographed on a single frame of film by delivering a series of strobe flashes (five per sec with an Olympus electronic flash T32) from above with the camera shutter open. To get a sharp focus, a narrow trough, 3.5 cm long, 1.0 cm wide and 2.5 cm deep, was used with the camera aimed at its long side. ST's were induced by illuminating the long axis of the trough horizontally. To study QTA's, a second light was added on the opposite side of that used for ST. Here, the two light sources used were 100 V-30 W incandescent bulbs which provided, respectively, 6.0 and 55 $\mu W/mm^2$ at the front surface of the trough. After illuminating the worm to be tested with the two beams for more than 3 min, QTA was induced by extinguishing the brighter light.

To investigate the ultrastructure of the eyes, worms were fixed at room tem-

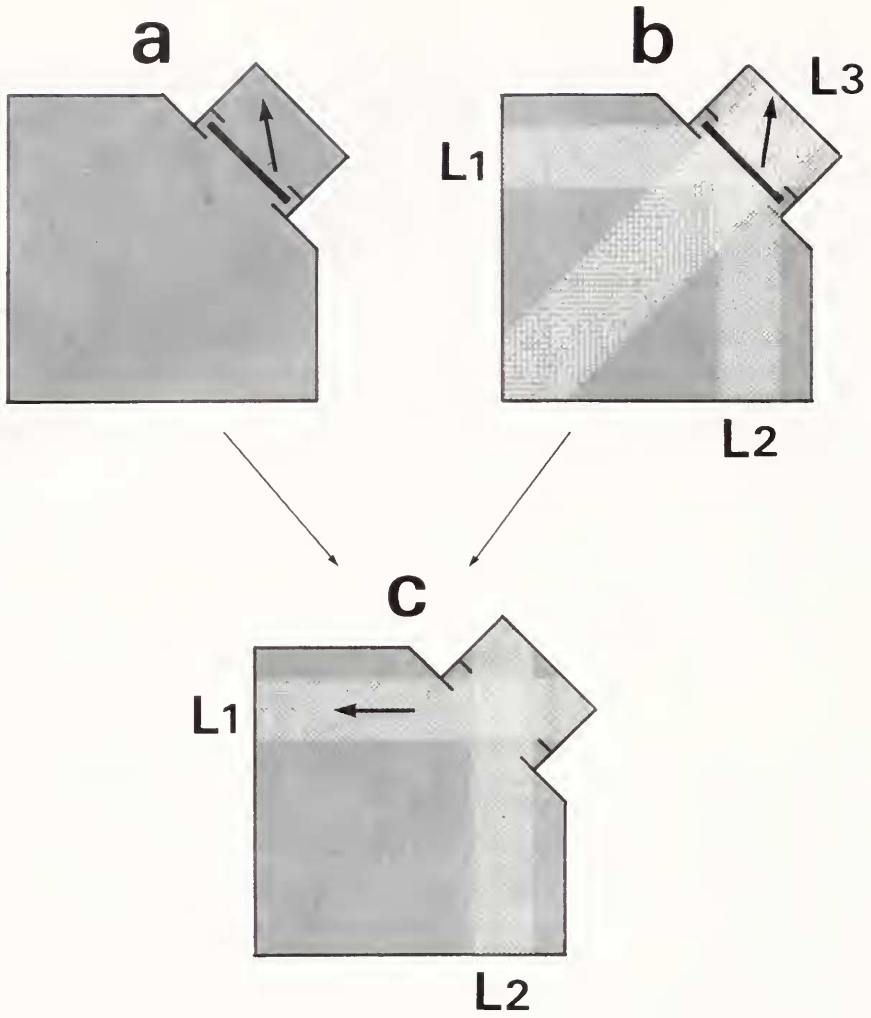

FIGURE 1. Experimental procedure to induce QTA and ST visual response (see text in detail).

perature for 2 h in 2.5% glutaraldehyde and post-fixed for 1 h in 1% osmium tetroxide, both being buffered to pH 7.4 by 0.1 *M* cacodylate containing 0.4 *M* sucrose. After dehydration through a graded series of ethanol, the specimens were embedded in the epoxy resin. Semi-serial EM sections were cut in three planes (transverse, sagittal and horizontal) on a Porter-Blum MT2 ultramicrotome, stained with alcoholic uranyl acetate and lead citrate and examined in a Hitachi H 500H electron microscope.

RESULTS

Orientation of worms responding to light

Sagitta has a pair of large seminal vesicles located laterally to the body axis just anterior to the caudal fin. These appear quite differently when viewed at various

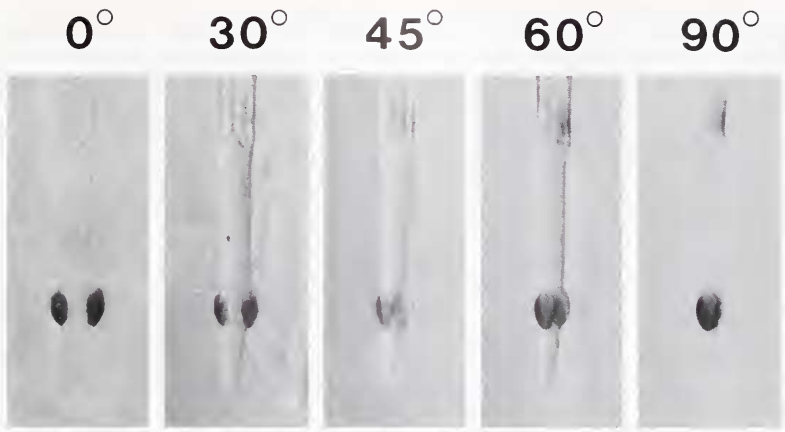

FIGURE 2. Appearance of seminal vesicles viewed from different angles relative to the dorso-ventral axis at 0°. Photographs taken from different angles (0°, 30°, 45°, 60° and 90°) on an agar plate with a narrow and shallow ditch.

angles to the dorso-ventral plane (Fig. 2). Orientations of profiles of swimming worms recorded in strobe flashes can be determined by refering to the appearance of the seminal vesicles. Since the precision of angular inclinations on the pictures was limited, we classified the profiles as dorso-ventral, oblique and lateral orientations, when the seminal vesicles appeared in separation, just attached to each other and overlapped, respectively (Table I).

Four examples are shown in Figure 3 in which a and b are QTA responses. Note that *i*) lateral profiles are seen in the bending phase (both in a and b), *ii*) quickly swimming worms keep the dorso-ventral plane horizontally (profiles on the right in a and one profile on the right in b) and *iii*) the worm in b, which oriented in the opposite direction against the light source, did show the QTA response. When sinking passively, however, the dorso-ventral axis gradually changed from more or less horizontal to vertical. During an ST response lateral and oblique profiles were recorded (Fig. 3c). The abrupt shift in position without a bending profile shown from left three profiles to the middle group in Figure 3c indicates the rapidity of the bending stroke occurring between strobe flashes delivered at 0.2 sec intervals.

The relation of *Sagitta*'s dorso-ventral plane, hence the orientation of eyes, with respect to the light source was studied by taking pictures while the animal was either approaching the light source by ST or was stimulated to swim by QTA. Table I summarizes the results of changes in orientation of the dorso-ventral plane, determined by using two or three successive profiles. Binomial tests were made to reveal whether differences observed between dorso-ventral and lateral orientations in each phase of reaction were significant or not.

Several important implications are evident. First, most of the bending worms (Table I b) were recorded as lateral profiles. Hence the body's motion in swimming strokes apparently occurs dorso-ventrally. In other words, the quick swimming is achieved not by an eel-like lateral stroke but by a dolphin-type thrust. Second, during the gliding phase (Table I c and d) after the first stroke most intact worms orient their dorso-ventral plane horizontally. Although one-eyed worms seem to show the same tendencies in Table I, the differences between dorso-ventral and lateral orientation were not statistically significant. Third, orientation of the dorso-ventral axis was random under all the other conditions. The last result implies that i) regardless

TABLE I

The number of incidences of orientations around the antero-posterior axis when the worms were in varying phases of motions in response to horizontally directed light and in passively sinking phases

The motion of the worm	Worm's eye	Orientations			Binomial test*
		Dorso-ventral	Oblique	Lateral	
QTA					
a. immediately before bending	intact	6	1	9	NS
	one eye	2	0	0	NS
b. in bending	intact	3	5	26	$P < .001$
	one eye	0	1	6	$P < .05$
c. immediately after bending	intact	23	4	7	$P < .001$
	one eye	5	1	1	NS
d. reached the lit wall	intact	20	3	8	NS
	one eye	7	0	2	NS
ST					
e. immediately before bending	intact	14	9	5	$P < .05$
	one eye	14	6	11	NS
f. immediately after bending	intact	15	4	9	NS
	one eye	22	5	4	$P < .001$
control					
g. passively sinking	intact	6	2	11	NS

* Statistical significance of difference between dorso-ventral and lateral orientations.

The orientations recorded by a camera aimed at the long side of a trough were determined by the appearance of seminal vesicles.

of the initial body orientation (Fig. 3a and b, Table I a), the worms can locate a visual target in QTA responses and are correctly aligned within a fraction of a second, ii) in ST responses, more frequent tilting of the antero-posterior body axis toward the light source (Goto and Yoshida, 1981) can be achieved under any orientation of the dorso-ventral plane (Table I f), and iii) when the chaetognaths are sinking passively, their dorso-ventral plane is randomly oriented (Table I g). The results obtained with one-eyed worms approaching the target by ST movements may suggest a preferred orientation of the dorso-ventral plane for the visually defective worms to locate a light source (Table I f).

A few comments may be added as regards the item (i). The data presented here are all concerned with rotation around the long axis of the body. One may ask the importance of the vertical inclination of the long axis. A part of the answer may be found in Figure 3b in which the worm oriented in the opposite direction against the target to go. Re-examination of the data which we accumulated for publication of a single photograph in our previous paper (Fig. 5 in Goto and Yoshida, 1981) revealed that 51 out of 71 worms had oriented in the opposite direction against the target before they responded by QTA's. This result does not necessarily mean that the opposite direction was preferable for the animal to locate that target but was simply due to our experimental procedure (cf. Fig. 7 in Goto and Yoshida, 1981)

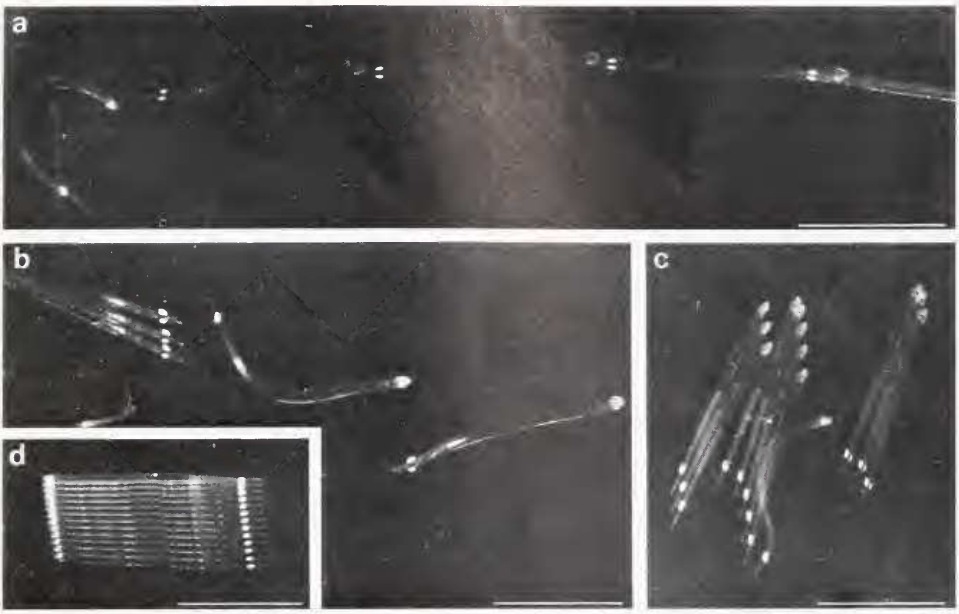

FIGURE 3. Examples of profiles of *Sagitta* showing QTA responses (a and b), ST response (c) and passive sinking (d). The target light source was to the right. Scale bars indicate 0.5 cm. a: The bending profile of stroke to the left is followed by gliding profiles with horizontal dorso-ventral plane. b: Passively sinking worm to the left points to the right with a bending profile in the middle. c: Three shots to the left and five in the middle show lateral profiles and three to the right show oblique ones. d: The dorso-ventral plane is more or less horizontal in the uppermost profile and vertical in the lowest profile.

whereby the animals were accumulated in one end of the trough opposite to the target by their ST movements.

Effect of selective microcauterization

Behavioral results obtained with the worms which were cauterized in varying ways are summarized in Table II and light micrographs of the cauterized eye regions are shown in Figure 4. Nine out of 10 of the intact control worms which recovered from anesthetization (Fig. 4a) showed both types of normal response. One worm failed to show QTA. Hence the eugenol exposure was without significant aftereffect. Sham control experiments were made by cauterizing the epidermis covering the area between the two eyes (Fig. 4b). Though some of the microcauterized worms became unresponsive, more than 60% of the 18 worms concerned responded normally (b in Table II). Considering the delicacy of their body structure, the ability of *Sagitta* to retain responsiveness after local cauterization may be considered high.

Worms in which one eye (d in Fig. 4 and Table II) and some of the epidermis above it (c in Fig. 4 and Table II) were cauterized resembled the sham control worms in their responses. Cauterization at two sites (e in Fig. 4 and Table II) increased the number of unresponsive worms, but only when both eyes were completely destroyed (f in Fig. 4 and Table II) did the microcauterized worms lose all their responsiveness to light. Even so, they could still swim in a disoriented manner.

We next selectively cauterized the brain and the ventral nerve ganglion on the trunk which lie just under the epidermis at some distance anterior and posterior to the eyes, respectively (g and h in Table II). Surprisingly, worms with a damaged

TABLE II

Effects of microcauterization of various tissues upon two types of light-oriented responses of Sagitta crassa

Microcauterized tissue	ST		QTA		
	+	−	++	+	−
a. none (control)	10	0	7	2	1
b. epidermis between two eyes	11	7	12	2	4
c. epidermis above one eye	12	5	8	3	6
d. one eye	13	7	7	4	9
e. epidermis above two eyes	3	6	2	0	7
f. both eyes	0	5	0	0	5
g. brain	0	4	0	0	4
h. ventral ganglion	0	3	0	0	3

Intact worms experienced anesthetization before experiments. One plus for slow-tactic responses (ST); the worm tested reached the wall facing either one of the light sources within 10 min. Two pulses for quick target-aiming (QTA); the worm tested swam rapidly and reached one of the lit walls. One plus (QTA); the worm tested simply became more active. Minus (ST on QTA); "no response". The figures indicate the number of worms which showed the respective type of responses.

brain showed the normal 'hop and sink' swimming pattern, but they did not respond to light even though both eyes were intact. On the other hand, worms with a damaged ventral ganglion but with the brain left intact no longer swam but sank passively down to the bottom.

Eye structure

Chaetognaths are known to possess a pair of lensless eyes under the epidermis (as shown in Fig. 4a) of the head region. Each eye consists of four kinds of cells; capsule cells which surround the external surface of the eye, one centrally located pigment cell surrounded by photoreceptor cells of an inverted ciliary type and glial cells among the photoreceptor units.

It has been held that the pigment cell of each eye in *Sagitta hexaptera* (Hesse, 1902), *S. bipunctata* (Burfield, 1972) and *S. scrippsae* (Eakin and Westfall, 1964) have five depressions. The present data on *S. crassa* reveal (Fig. 5) that the pigment cell at the center has seven depressions on four sides, *e.g.*, one anterior, one posterior, one lateral and four equal medial ones. A simplified model is shown in Figure 5d. Visual cells around the pigment cell extend into its depressions distal endings which presumably comprise the photoreceptive site (Eakin and Westfall, 1964; Ducret, 1978). Light coming from one particular direction will be attenuated largely by pigment granules. Though incasement by depressions of the pigment cell is not complete, the amount of light absorbed by the distal endings will inevitably be different among seven groups of sensory cells. Semi-serial sections revealed that the proximal ends of the sensory cells extended as optic nerve fibers (Fig. 6a) anteriorly to the brain. There were about 90–100 axons in transverse sections of the optic nerve (Fig. 6b) but no synapses were found between the eyes and the brain. This number of axons is rather small compared with *S. scrippsae* which has 500–600 (Eakin and Westfall, 1964).

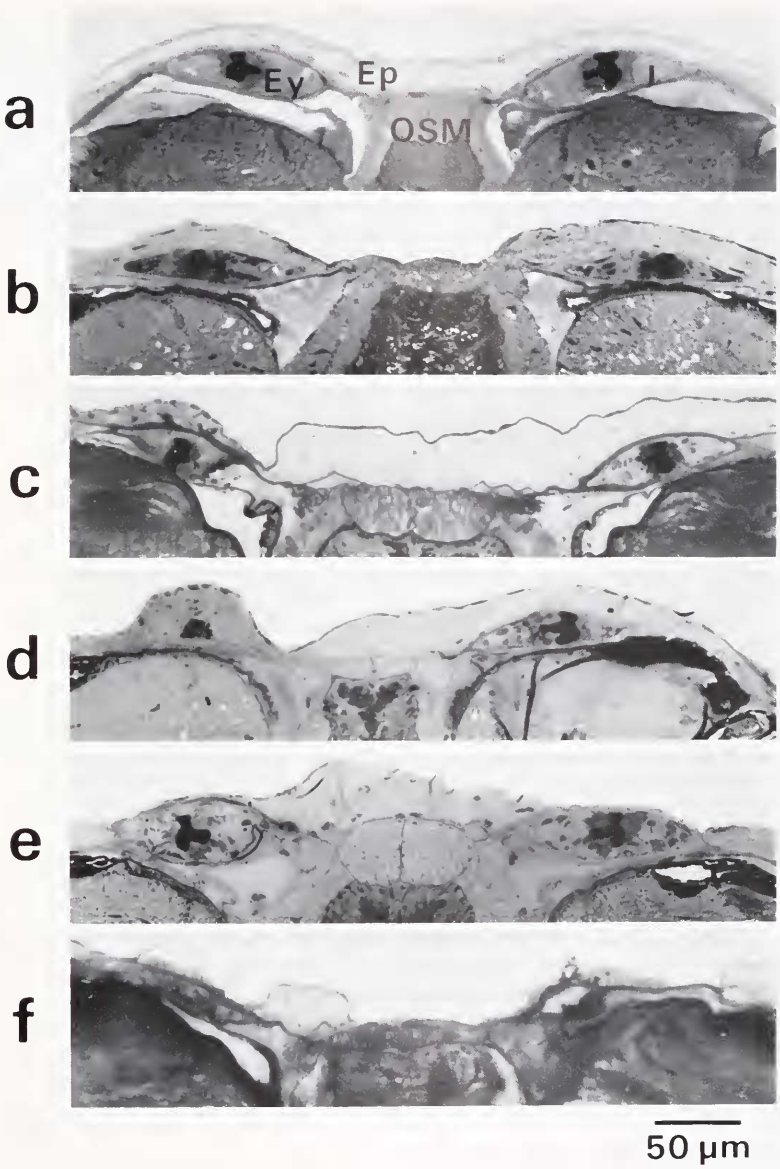

FIGURE 4. Light microscopic profiles of the ocellar region of *Sagitta*. a: Intact. b: Sham control with epidermis injured between the two eyes. c: Epidermis above left eye injured. d: Left eye damaged. e: Epidermis above both eyes injured. f: Both eyes completely damaged. Ep, epidermis; Ey, eye; OSM, oblique superficial muscle.

DISCUSSION

When arrow worms were responding to two identical beams of light either by ST or by QTA movements, none of the worms neither intact nor sham-operated, took an intermediate path between the two beams. Following the terminology de-

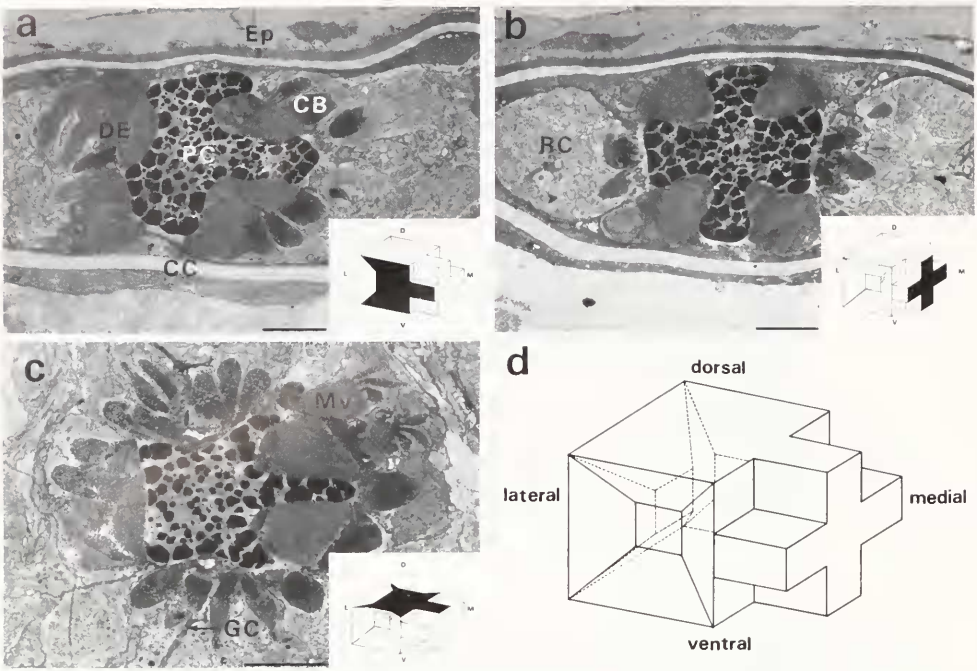

FIGURE 5. Electron micrographs of transverse (a), sagittal (b) and horizontal (c) profiles of one eye and schematic representation of the pigment cell (inserts and d). CB, conical body; CC, capsule cell; DE, distal ending of the photoreceptor cell; Ep, epidermis; GC, glia cell; Mv, microvilli; PC, pigment cell; RC, receptor cell. Scale bars indicate 5 μm.

fined by Frankel and Gunn (1961), both types of photic responses may be referred to as telotaxis since both eyes were not necessary for the oriented responses. Our microcauterization experiments demonstrated that the presence of one eye was sufficient for the worms to swim accurately toward the light source (Table II).

There is no direct physiological evidence that the so-called eyes of arrow worms are in fact photoreceptors, though this seems likely from what is now known to their morphology (Hesse, 1902; Burfield, 1927; Eakin and Westfall, 1964; Ducret, 1978). The present experiments prove that arrow worms lose all responsiveness to light without both eyes. This means that the eyes are the photoreceptive sites for the two light responses studied and that no extraocular photoreceptors can be substituted for them.

When microcauterization injured only the epidermis above the two eyes, some worms still showed light responses even though the light reaching the eye from the normal direction was presumably attenuated largely by the damaged epidermis. This indicates that effective light stimuli for target location can pass through the transparent body from other directions.

Hyman (1959) wrote: "The forward dart is so rapid as to be difficult of analysis but is believed to result from the alternate contractions of the dorsal and ventral longitudinal muscle bands of trunk and tail". The dorso-ventral bendings recorded at the time of swimming strokes clearly show involvement of those muscle bands. When gliding rapidly toward the light source after the first bending stroke, however, worms rotate around their antero-posterior axis by 90° so that the dorso-ventral axis is horizontal. The resulting vertical orientation of two pairs of the lateral fins

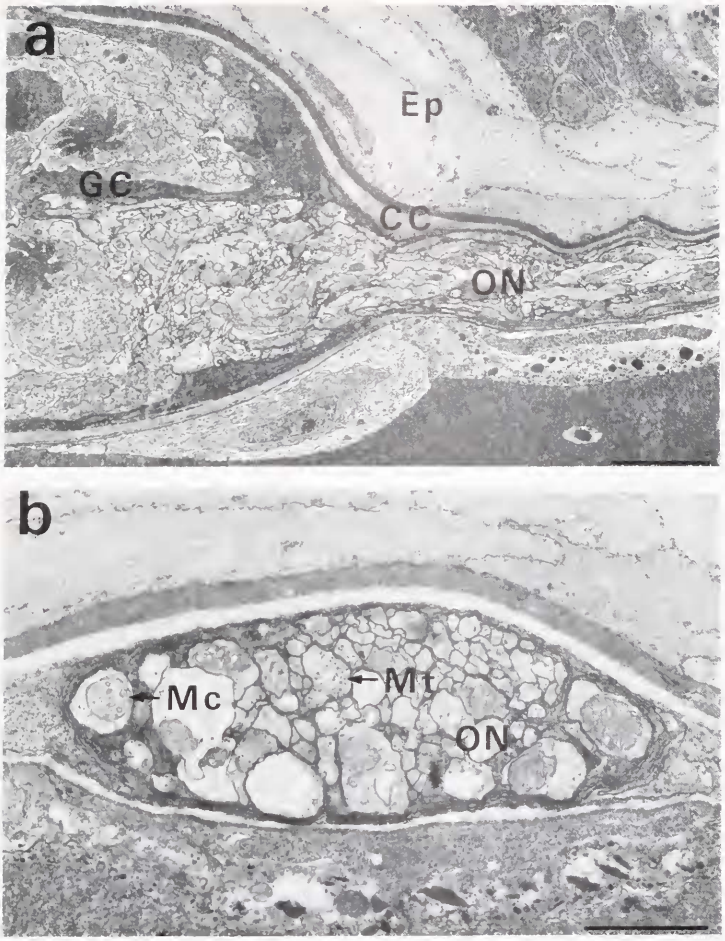

FIGURE 6. Electron micrographs of sagittal (a) and transverse (b) profiles of a bundle of optic nerves. In a, the eye is to the left. CC, capsule cell; Ep, epidermis; GC, glial cell; Mc, mitochondria; Mt, microtubule; ON, optic nerve. Scale bars indicate 5 μm in a and 2 μm in b.

may be advantageous for the worms to keep the target-aiming path during the course of gliding phase.*

The fact that the worm can locate a light source regardless of its orientation either to the dorso-ventral or to the antero-posterior axis can probably be explained by the structure of the eye. As described above, the eye of the arrow worms has photoreceptive endings which are separated into seven groups by the centrally located pigment cell. Due to its shielding effect, a particular group of the endings will receive light coming from a particular direction more effectively than the others. Synapses are lacking between sensory cells and along the optic nerve. A specific combination of excitation pattern which depends on a corresponding direction of the incident beam may be processed in the brain as an information to locate the target. Combined with the results obtained by injuring the brain and the ventral

* We thank one of the referees for this suggestion.

ganglion, the latter may be assumed to be a locomotor center in which responses to light are controlled by the eyes through the brain.

The effect of water borne vibration in inducing QTA movements and sensing of the inclination angles of the antero-posterior axis in the ST response (Goto and Yoshida, 1981) lead one to expect some kind of mechanoreceptors. Cilia distributed over the body surface may be part of such a system (Burfield, 1927; John 1933; Horridge and Boulton, 1967; Bone and Pulsford, 1978). Detailed analyses and hopefully recordings of the ventral ganglion's input and output pathways as well as those of the brain, are needed to improve our understanding of the unique behavioral patterns of chaetognaths.

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